IL-6–174 G/C and ApoE Gene Polymorphisms in Alzheimer's and Vascular Dementia Patients Attending the Cognitive Disorder Clinic of the All India Institute of Medical Sciences, New Delhi

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Key Words

Alzheimer's disease • Apolipoprotein E • Dementia • Interleukin-6 • Neurodegenerative disease • Polymorphism • Vascular dementia

Abstract

Background: Remarkable improvement in the life expectancy of the Indian population is expected to commensurate with the increase in number of dementia cases. Among various types of dementia, Alzheimer's disease (AD) and vascular dementia (VaD) are common and widely studied. We evaluated the role of apolipoprotein E (ApoE) and interleukin-6 (IL-6)–174 G/C gene polymorphism along with serum IL-6 levels in AD and VaD patients. **Methods:** The polymorphisms in ApoE and IL-6–174 G/C genes were assessed using RFLP. Serum IL-6 level was measured by ELISA. **Results:** The allele ϵ 4 of the ApoE gene was found to be associated with AD and VaD patients ($p < 0.05$). No association of IL-6–174 G/C polymorphism was observed in AD patients, while the IL-6–174 C allele increased the odds of having VaD twofold. Regression analysis to assess possible interaction between ApoE and the IL-6–174 G/C genes revealed that presence of both the ϵ 4 and C alleles increased the odds of having AD 13.75-fold and VaD 14.7-fold. Serum IL-6 levels did not correlate with either

presence or severity of disease among AD or VaD patients.

Conclusion: The ApoE ϵ 4 allele is an important genetic marker for AD and VaD. Presence of both ApoE ϵ 4 and IL-6 C genes increases the OR of having AD and VaD markedly.

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Introduction

Dementia is defined as a decline in mental abilities and cognitive functions which eventually impairs the ability of an individual to carry out everyday activities. The 2 most common types of dementia are Alzheimer's disease (AD) and vascular dementia (VaD).

As per the 2005 estimate of the World Health Organization (WHO), nearly 0.379% of the world's population had dementia and this will further increase to 0.556% by the year 2030. Well-conducted studies in India [1] and China [2] suggest similar prevalence rates for dementia as seen in the developed countries. A recent study conducted by Kalaria et al. [3] reported that the percentage prevalence of AD and VaD among adults >65 years of age in

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India is nearly 1.3 and 1.1%, respectively. Considering India's increasing life expectancy and population of nearly 70 million elderly persons, the number of dementia cases is expected to go up, and as a result, the demands for health care of demented people will rise remarkably in this century [4].

AD is characterized by progressive cognitive deterioration with declining daily living activities and neuropsychiatry symptoms. In AD, the pathological process consists principally of neuronal loss or atrophy, mild inflammatory response with deposition of amyloid, and formation of neurofibrillary tangles and β -amyloid plaques [5]. This neurodegenerative disease has a multifactorial etiology involving complex interactions of both genetic and environmental factors. VaD results from heterogeneous brain lesions caused by ischemia, hypoperfusion or hemorrhage of cerebral tissue. The pathophysiology of VaD is complex and involves a number of mechanisms, including reduction of cerebral blood flow, alteration of oxygen metabolism and inflammatory mechanisms.

Genetic factors have been widely proven to be associated with both AD and VaD. In sporadic AD, many candidate genes are involved in the metabolism of β -amyloid [α -2-macroglobulin, cystin C, low-density lipoprotein-related protein 1, apolipoprotein E (ApoE), cathepsin D, etc.] or protein which mediates the AD-related immune system reaction like interleukin (IL)-1 α and IL-6 [6]. ApoE has been widely studied and is a well-established risk factor for AD. Out of 3 alleles of ApoE, ϵ 4 allele has been associated with an increased risk of AD [7–11]. ApoE ϵ 4-targeted replacement in mice demonstrated a proinflammatory phenotype including increased iNOS mRNA synthesis and NO production, and higher proinflammatory cytokine production (TNF- α , IL-6, IL-12p40) in glial cell culture in comparison to the ApoE ϵ 3-targeted replacement mice [12]. Several studies in the past have been conducted to find an association of the IL-6–174 G/C polymorphism with AD, but the results have been inconsistent. Studies conducted across varying geographical areas have yielded different results: some have shown a positive association [13–18], while others have found none [19–26]. Additionally, serum IL-6 levels have also been reported to be increased in some studies [13, 14], while in others no significant differences were observed in comparison to the healthy controls [27, 28].

India has a vast prevalence of the disease as mentioned earlier, and this fact has gone almost unnoticed. The major objective of the current study was to determine whether ApoE and/or IL-6–174 G/C polymorphism have any

association with AD and VaD, and also whether there is any association of serum levels of IL-6 with polymorphism of IL-6–174 G/C genes (rs 1800795) in the Indian population.

Patients and Methods

Patients

All patients coming to the cognitive disorder clinic at All India Institute of Medical Sciences (AIIMS), New Delhi, during November 2007 to February 2010 were screened for dementia. The patients were identified as having dementia by DSM-IV, Mini-Mental State Examination (MMSE score ≤ 24 out of 30) and detailed neurological examination [29]. Further, NINCDS-ADRDA and NINDS-AIREN were used for classification of AD and VaD. A total of 74 patients with AD and 46 with VaD were recruited for the present study. The age group of the patients recruited was ≥ 50 years. A detailed pedigree chart was drawn for all patients and none was found to have family history. All patients having any reversible cause of dementia like vitamin B₁₂ deficiency, hypothyroidism or neuro-infections causing dementia (neurosyphilis, HIV, tuberculosis) and rapidly progressive dementia (dementia evolving and progressing from stage I to stage III in ≤ 6 months) were excluded from the current study. Magnetic resonance imaging (MRI) of the brain was done in all patients to confirm the diagnosis.

Healthy Controls

A total of 113 healthy controls (of comparable age and gender matched ± 3 years) were recruited and their MMSE scores were determined. All the healthy controls that had MMSE scores of ≤ 24 or a history of disease (cardiovascular, stroke, diabetes, AIDS or tuberculosis) were excluded. This study protocol was approved by the ethics committee of AIIMS. Consent for the study was obtained from all the enrolled subjects.

Sample Collection

Approximately 6 ml of blood was collected from fasting subjects in the supine position and was drawn by puncture of the antecubital vein. The drawn blood was equally distributed in plain and EDTA vials for biochemical and genetic studies, respectively.

DNA Extraction

Genomic DNA was extracted from the EDTA-treated blood by the salting out method [30]. The yield of the extracted DNA was then measured at 260 nm using a spectrophotometer and the purity was calculated using the 260/280-nm ratio. All DNA samples were stored at -20°C until further analysis.

ApoE Genotyping

Amplification of the ApoE gene-containing region of the DNA was amplified in a Mastercycler Ep Gradient Thermocycler (Eppendorf AG, Hamburg, Germany) using 100-ng of the genomic DNA. The ApoE gene was amplified using previously published primers [31]. Briefly, 25 μl of PCR product was generated using 100 ng genomic DNA, 10 $\times$ reaction buffer, 0.2 mM deoxynucleotide triphosphates, 15 mM MgCl₂, 20 pmol of each primer and 1.5 units of Taq DNA polymerase (New England Biolabs, USA). The

The PCR profile involved initial denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 65°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 7 min. The amplified product was electrophoresed on a 2% agarose gel. Further genotyping was done following digestion of 10 µl of the amplified product using 5 units of HhaI restriction enzyme (New England Biolabs) and incubated at 37°C for 5 h. The digested product was further separated on an 8% polyacrylamide gel and visualized by silver staining.

IL-6–174 G/C Genotyping

For amplification of the IL-6–174 G/C (rs 1800795), the gene-containing region of the DNA was amplified using previously published primers [32]. Briefly, 25 µl of PCR product was generated using 100 ng of genomic DNA, 10× reaction buffer, 0.2 mM of deoxynucleotide triphosphates, 20 pmol of each primer and 1.5 units of Taq DNA polymerase. The PCR profile involved initial denaturation at 95°C for 10 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. The amplified product was electrophoresed on a 2% agarose gel. Further genotyping was done following digestion of 10 µl of the amplified product using 2 units of SfaNI restriction enzyme and incubation at 37°C for 5 h. The digested product was further separated on a 2.5% agarose gel and visualized under a transilluminator.

Estimation of IL-6

The levels of IL-6 cytokine in serum of all patients and controls were determined using a commercially available ELISA kit (Ready-SET-Go! Human Interleukin-6, eBioscience, USA) as per the manufacturer's protocol. The intra- and interassay coefficients of variation were 2.8–5.4 and 4.1–7.9%, respectively.

Statistical Analysis

All statistical analysis was done using SPSS, version 17.0. The descriptive analysis, mainly means and SD for age, sex, onset of disease and other clinical symptoms, was calculated for all patients and controls. Differences in genotype and allelic distribution among the diseased and healthy controls were determined using a χ^2 test, with the OR and 95% CI calculated subsequently. Multinomial logistic regression was also done to examine the interaction between ApoE and the IL-6–174 G/C alleles.

Results

In this case control study, a total of 233 subjects, which included 74 AD, 46 VaD and 113 controls, were studied for polymorphism in ApoE and the IL-6–174 G/C genes. Serum IL-6 was also measured in all subjects.

Demographic and Clinical Profile of the Subjects

Table 1 shows the demographic and clinical profile of the control and the patients with AD and VaD. There was no significant difference in the mean age group of AD (66.4 ± 9.2) or VaD (66.1 ± 8.8) in comparison to the control subjects (64.0 ± 8.4). However, there was a rela-

Table 1. Demographic and clinical characteristics of the 3 study groups

	AD (n = 74)	VaD (n = 46)	Healthy controls (n = 113)	p
Age ^a (in years)	66.4 ± 9.2	66.1 ± 8.8	64.0 ± 8.4	>0.05 ^{b, c}
Onset of disease ^a (in years)	63.2 ± 9.3	63.6 ± 8.2	–	>0.05 ^d
Sex				
Male	50	34	72	>0.05 ^{b, c}
Female	24	12	41	
MMSE	17.2 ± 4.6	17.4 ± 4.7	–	>0.05 ^d
BDR	13.6 ± 4.2	13.4 ± 4.2	–	>0.05 ^d
IADL	19.4 ± 7.4	16.7 ± 6.8	–	0.041 ^d

BDR = Blessed dementia rating; IADL = incremental activities of daily living.

^a Values are represented as means ± SD.

^b AD vs. healthy controls.

^c VaD vs. healthy controls.

^d AD vs. VaD.

tively low percentage of females in the AD (32.4%) and VaD (26.1%) groups, which was not significantly different from the control group. One interesting feature is that out of 74 AD cases, 17 patients (11 male and 6 female) were below 60 years of age.

The means with SD of MMSE, BDR and IADL for AD and VaD patients were 17.2 ± 4.6 and 17.4 ± 4.7 , 13.6 ± 4.2 and 13.4 ± 4.2 , 19.4 ± 7.4 and 16.7 ± 6.8 , respectively (table 1). Significant differences among the mean incremental activities of daily living levels were observed within the AD and VaD groups ($p = 0.041$), while the levels of MMSE and the Blessed dementia rating were not significantly different.

Allele Distribution and Genotyping of ApoE Genes in AD and VaD

Table 2 shows the ApoE allele distribution and genotyping found in AD and VaD patients as compared to controls. The frequency of ApoE alleles $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ were examined in 233 subjects. Allele $\epsilon 2$ was absent in AD patients and was present in only 1 case of VaD patients as compared to 7 out of 113 control subjects. Allele $\epsilon 3$ was present in 90.3% of the controls, 73% of the AD cases and 81.5% of the VaD cases. The frequency of allele $\epsilon 4$ was found to be significantly high in the AD (27%) and VaD (17.4%) cases as compared to control subjects (6.6%). Compared to the $\epsilon 3$ allele, the $\epsilon 4$ allele was more frequent

Table 2. Allele and genotype distribution of the ApoE gene among diseased and healthy control groups

	AD (n = 74)	VaD (n = 46)	Healthy controls (n = 113)	p
Alleles				
ε2	–	1.1 (1)	3.1 (7)	0.045 ^a >0.05 ^b
ε3	73.0 (108)	81.5 (75)	90.3 (204)	<0.001 ^a 0.031 ^b
ε4	27.0 (40)	17.4 (16)	6.6 (15)	<0.001 ^a <0.003 ^b
Genotypes				
ε2ε2	–	–	–	–
ε2ε3	–	2.2 (1)	5.3 (6)	>0.05 ^{a, b}
ε2ε4	–	–	0.9 (1)	>0.05 ^{a, b}
ε3ε3	54.1 (40)	67.4 (31)	81.4 (92)	<0.001 ^a >0.05 ^b
ε3ε4	37.8 (28)	26.1 (12)	12.4 (14)	<0.001 ^a 0.034 ^b
ε4ε4	8.1 (6)	4.3 (2)	–	0.003 ^a >0.05 ^b

Figures in parentheses represent numbers.

^a AD vs. healthy controls.

^b VaD vs. healthy controls.

among AD ($p < 0.001$) and VaD cases ($p = 0.031$) as compared to controls. The presence of the ε4 allele increased the odds of having AD sixfold (95% CI: 2.9–12.4) and 3.1-fold in VaD (95% CI: 1.3–7.2). In the younger group of females (6 cases), the ApoE ε4 allele frequency was double (66%) that of females over 60 years of age (33%).

The frequency of 6 genotypes, ε2ε2, ε2ε3, ε2ε4, ε3ε3, ε3ε4 and ε4ε4, are shown in table 2; the frequency of genotype ε3ε4 was higher in AD (37.8%) and VaD (26.1%) patients than in control subjects. Genotype ε4ε4 was more frequent in AD (8.1%) and VaD (4.3%), while absent in control subjects.

Within the genotypes, genotype ε3ε4 was frequently detected among the AD ($p < 0.001$) and the VaD cases ($p = 0.034$) in comparison to the healthy individuals, while the percentage of the ε4ε4 genotype was significantly higher within the AD group ($p = 0.004$). In addition, the ε3ε3 genotype was detected less frequently among the AD group in comparison to the healthy controls ($p < 0.001$).

Table 3. Allele and genotype distribution of IL-6–174 G/C gene among diseased and healthy control groups

	AD (n = 74)	VaD (n = 46)	Healthy controls (n = 113)	p
Alleles				
G	83.8 (124)	73.9 (68)	85.0 (192)	>0.05 ^a 0.021 ^b
C	16.2 (24)	26.1 (24)	15.0 (34)	
Genotypes				
GG	68.9 (51)	54.4 (25)	72.6 (82)	>0.05 ^a 0.026 ^b
GC	29.7 (22)	39.1 (18)	24.8 (28)	>0.05 ^{a, b}
CC	1.4 (1)	6.5 (3)	2.6 (3)	>0.05 ^{a, b}

Figures in parentheses represent numbers.

^a AD vs. healthy controls.

^b VaD vs. healthy controls.

Allele Frequency and Genotype of IL-6–174 G/C Genes in AD and VaD

From the data as shown in table 3, it is clear that there was no significant difference in distribution of allele frequency G in AD (83.8%) and VaD cases (73.9%) in contrast to the control subjects (85%).

However, there was a significant difference of frequency of C allele in VaD cases (26.1%) as compared to control subjects (15%), while no such difference was found in AD cases (16.2%). Compared to the G allele, the C allele was more frequent among VaD cases ($p = 0.021$) as compared to the controls. The absence of the G allele increased the odds of having VaD 2.2-fold (95% CI: 1.03–4.82).

Of the 3 genotypes GG, GC and CC, the last one (CC) was less frequent in controls (2.6%) as compared to AD (1.4%) and VaD (6.5%), while the first one (GG) was found more frequently. While no correlation of genotype GG was found in the AD patients, there was a statistically significant correlation ($p = 0.026$) between the controls and VaD patients.

Probability of Interaction of ApoE and IL-6–174 G/C Gene in Causation of AD and VaD

To assess the interplay of ApoE and the IL-6–174 G/C genes in causation of AD and VaD, multinomial logistic regression analysis was done. In absence of the ε4 allele, the IL-6 C allele alone did not have any significant causal relationship with either AD ($p = 0.93$) or VaD cases ($p =$

Table 4. Regression analysis to show OR for AD and VaD in interaction between ApoE and IL-6-174 G/C alleles

ApoE ϵ 4	IL-6 C	Healthy controls	AD	VaD	p	OR (95% CI)
-	-	70	28	19		1 (reference)
-	+	29	12	13	0.93 ^a	1.03 (0.46–2.30) ^c
					0.23 ^b	1.65 (0.72–3.78) ^d
+	-	12	23	6	<0.001 ^a	4.79 (2.10–10.92) ^c
					0.28 ^b	1.84 (0.61–5.55) ^d
+	+	2	11	8	0.001 ^a	13.75 (2.86–66.03) ^c
					0.001 ^b	14.74 (2.89–75.24) ^d

+/- = Presence/absence.

^a AD vs. healthy controls. ^b VaD vs. healthy controls. ^c AD and healthy controls. ^d VaD and healthy controls.

0.23). The association with AD cases was significant ($p < 0.001$) in the presence of the ϵ 4 allele and in absence of the IL-6 C allele, while for VaD cases such an association was statistically insignificant. The presence of both the ApoE ϵ 4 and IL-6 C alleles had a positive modifier effect, increasing the OR of having AD 13.75-fold and 14.74-fold for VaD.

Serum Levels of IL-6

The serum IL-6 levels determined in all 3 groups (AD, VaD, controls) are shown in figure 1. The median (range) of IL-6 (pg/ml) levels among the healthy controls, AD and VaD groups were 2.91 (0–24.85), 3.82 (0–32.56) and 4.49 (0–23.8), respectively (fig. 1). The Kruskal-Wallis test revealed no significant differences in the IL-6 levels among the 3 groups ($p > 0.05$). No association between serum IL-6 level and IL-6 gene polymorphisms was observed. In 17 AD patients who were below 60 years of age, the median of serum IL-6 level was 3.61 (0–6.78) as compared to 57 patients above 60 years of age whose median value was 3.92 (0–32.56). Therefore, the present study does not show any statistically significant rise of IL-6 with advancement of age. Twenty-four samples (12 within the healthy control group and 6 each within the AD and VaD groups) did not have detectable IL-6 levels.

Discussion

The present study has shed light on epidemiological and etiopathological aspects of 2 predominant dementias, AD and VaD, diagnosed clinically and confirmed with the help of MRI at AIIMS.

Fig.1. Box plot showing serum IL-6 levels of the 3 study groups.

Concerning the epidemiological aspect, a higher ϵ 4 allele frequency (27%) was observed in the Alzheimer's group in comparison to the healthy controls (6.6%). The percentage prevalence of the ϵ 4 allele among the diseased populations worldwide, however, is not consistent. Studies from Norway and Spain [7, 8] have reported a higher frequency of the ϵ 4 allele, while relatively lower percentages have been reported from Hungary and Iran [33, 34]. Observations similar to our study have been reported in the past from Italy, Israel and China [9–11]. A recently conducted study involving neurodegenerative cases of dementia reported low frequency of the ϵ 4 allele (15%) in the Indian population [35]. The present study has shown that the presence of the ϵ 4 allele increases the risk of AD nearly sixfold, which clearly portrays causal linkage of the ϵ 4 allele of the ApoE gene with AD. Such an observation has been well established in earlier reported studies

as well. Among the genotypes, $\epsilon 3\epsilon 4$ was observed to be widely prevalent among the diseased groups in comparison to the healthy controls ($p < 0.001$), which is in accordance with earlier studies [36].

On the other hand, in the cases of VaD, the association of the $\epsilon 4$ allele is debatable. In the literature, one can find both an association [8–11] and no association [35–37] with ApoE polymorphism. In the present study, the frequency of the $\epsilon 4$ allele was significantly higher in cases of VaD patients (17.4%) in comparison to the control group ($p = 0.003$). The percentage prevalence of the $\epsilon 4$ allele was similar to studies from Hungary and China [11, 33]; however, one of the previous studies from our institute reported a relatively higher frequency of the $\epsilon 4$ allele in VaD patients [36]. The discrepancy could perhaps be due to the difference in samplings and/or relatively lower sample size.

In addition, the promoter region of IL-6 gene was also evaluated for assessment of the risk contribution to develop AD or VaD. Varying results have been reported in the literature in the context to polymorphism in the -174 G/C gene of IL-6. Studies conducted in Italy, Japan and Spain [13–15] showed a positive association of the G allele of IL-6 with AD, while other studies observed the C allele having a role in the pathogenesis of AD [16–18]. In the present study, we did not find any association of the G/C allele in the Alzheimer's group, which is in agreement with the previous reports published from the USA, Italy, Germany, UK, Finland and the Netherlands [19–26]. However, among the VaD patients, a significant association was found of the C allele with the diseased state ($p = 0.021$). To date, there are only limited reports in the literature that have evaluated the role of IL-6-174 G/C polymorphism. Among these, one of the studies from the Netherlands [25] did not find any association. Another study from Italy, in which patients with multi-infarct dementia were evaluated, reported an association of the G allele among the diseased population [38]. The present study has shown the association with the C allele. Similar studies which aim to address this issue in patients with VaD are thus important.

The present study also contributes to etiopathological issues as evident from age-related findings and the OR obtained by multinomial regression analysis. Of the 74 cases of AD, 17 patients (11 male and 6 female) were below the age of 60 years. In the younger group of females (6 cases), the frequency (66%) of the ApoE $\epsilon 4$ allele was found to be doubled that of females above 60 years (33%). The most interesting finding in the present study is that among $\epsilon 4$ allele-positive subjects, it was observed that the

absence of GG genotype increased the odds of having VaD 14.74-fold and AD 13.75-fold.

Besides the genetic study, serum IL-6 levels were also measured to evaluate their role in the diseased population. No association was found between the IL-6-174 G/C polymorphism and serum levels of IL-6. This observation is supported by previous studies which reported that the level of IL-6 in blood is not associated with AD [27, 28]. However, few isolated reports have found such an association [13, 14]. In our study, the levels of serum IL-6 were relatively higher in both the AD and VaD groups as compared to control subjects, but this alteration was not statistically significant.

Although once elevated, the serum IL-6 level remains at the same level for some time [39]; 1-time measurement of IL-6 as a biomarker of neurodegenerative disease has its own limitations, offering information only for that time. Additionally, serum levels of any biomarker, including IL-6, does not always reflect the situation within and around the neurons. A longitudinal study with several measurements of IL-6 may be of some help. Furthermore, IL-6 levels have been reported to increase with age [40, 41] and vary with nutrition [42, 43]. These facts may be taken into consideration when one tries to explain the presence or absence of such an association. In the present study, however, no statistically significant change in serum IL-6 level was seen with advancement of age.

Nevertheless, the raised serum IL-6 level, in spite of not having any history of vascular or inflammatory episode, merits further exploration. It may be attributed to either the environmental factors or higher frequency of the G allele among the Indian population [44]. However, the rise was present in the control group of comparable age as well.

In conclusion, we found a statistically significant association of ApoE $\epsilon 4$ allele among both the diseased groups (AD and VaD), and the association is stronger in AD than in VaD. Our study reveals for the first time that the C allele increased the odds of having VaD 14.74-fold and AD 13.75-fold. In addition, the present study shows an association of the C allele of IL-6-174 genes with VaD, while in the AD group no such association was observed. That the incidence of the $\epsilon 4$ allele is almost doubled in younger females (less than 60 years) is also a notable finding in AD patients.

The current study was carried out in a single tertiary care hospital in Delhi. To strengthen the hypothesis that genetic markers are superior to phenotypic markers for early diagnosis of AD/VaD, which may be useful for ear-

ly detection of the disease-prone subjects in the prevalent area, such a study should be conducted with a large sample size with a multicenter and multiethnic approach involving both hospital-based and community-based populations. The role of serum IL-6 and polymorphism of its genes in AD and VaD patients could be further elucidated by designing studies which evaluate the role of other inflammatory cytokine genes which have influence on the serum IL-6 level.

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References

- Shaji S, Promodu K, Abraham T, Roy KJ, Verghese A: An epidemiological study of dementia in a rural community in Kerala, India. *Br J Psychiatry* 1996;168:745–749.
- Zhang MY, Katzman R, Salmon D, et al: The prevalence of dementia and Alzheimer's disease in Shanghai, China: impact of age, gender, and education. *Ann Neurol* 1990;27:428–437.
- Kalaria RN, Maestre GE, Arizaga R, Friedland RP, Galasko D, Hall K, Luchsinger JA, Ogunniyi A, Perry EK, Potocnik F, Prince M, Stewart R, Wimo A, Zhang ZX, Antuono P, World Federation of Neurology Dementia Research Group: Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. *Lancet Neurol* 2008;7:812–826.
- Thakur MK: Alzheimer's disease: a challenge in new millennium. *Curr Sci* 2000;79:29–36.
- Saunders AM, Strittmatter WJ, Schmechel D, et al: Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* 1993;43:1467–1472.
- Papassotiropoulos A, Streffer JR, Hock C, Nitsch RM: Genetics of Alzheimer's disease. *Schweiz Arch Neurol Psychiatr* 2001;152:278–287.
- Sando SB, Melquist S, Cannon A, Hutton ML, Sletvold O, Saltvedt I, White LR, Lydersen S, Aasly JO: APOE ϵ 4 lowers age at onset and is a high risk factor for Alzheimer's disease; a case control study from central Norway. *BMC Neurology* 2008;8:9.
- Frank A, Díez-Tejedor E, Bullido MJ, Valdivieso F, Barreiro P: APOE genotype in cerebrovascular disease and vascular dementia. *J Neurol Sci* 2002;203–204:173–176.
- Orsitto G, Seripa D, Panza F, Franceschi M, Cascavilla L, Placentino G, Matera MG, Paris F, Capurso C, Solfrizzi V, Dallapiccola B, Pilotto A: Apolipoprotein E genotypes in hospitalized elderly patients with vascular dementia. *Dement Geriatr Cogn Disord* 2007;23:327–333.
- Chapman J, Wang N, Treves TA, Korszyn AD, Bornstein NM: ACE, MTHFR, factor V Leiden, and APOE polymorphisms in patients with vascular and Alzheimer's dementia. *Stroke* 1998;29:1401–1404.
- Katzman R, Zhang MY, Chen PJ, Gu N, Jiang S, Saitoh T, Chen X, Klauber M, Thomas RG, Liu WT, Yu ES: Effects of apolipoprotein E on dementia and aging in the Shanghai Survey of Dementia. *Neurology* 1997;49:779–785.
- Vitek MP, Brown CM, Colton CA: APOE genotype-specific differences in the innate immune response. *Neurobiol Aging* 2009;30:1350–1360.
- Shibata N, Ohnuma T, Takahashi T, Baba H, Ishizuka T, Ohtsuka M, Ueki A, Nagao M, Arai H: Effect of IL-6 polymorphism on risk of Alzheimer disease: genotype-phenotype association study in Japanese cases. *Am J Med Genet* 2002;114:436–439.
- Mateo I, Infante J, Rodríguez E, Berciano J, Combarros O, Llorca J: Interaction between dopamine beta-hydroxylase and interleukin genes increases Alzheimer's disease risk. *J Neurol Neurosurg Psychiatry* 2006;77:278–279.
- Pola R, Flex A, Gaetani E, Lago AD, Gerardino L, Pola P, Bernabei R: The -174 G/C polymorphism of the interleukin-6 gene promoter is associated with Alzheimer's disease in an Italian population [corrected]. *Neuroreport* 2002;13:1645–1647.
- Licastro F, Grimaldi LM, Bonafe M, Martina C, Olivieri F, Cavallone L, Giovannetti S, Masliah E, Franceschi C: Interleukin-6 gene alleles affect the risk of Alzheimer's disease and levels of the cytokine in blood and brain. *Neurobiol Aging* 2003;24:921–926.
- Arosio B, Trabattoni D, Galimberti L, Bucciarelli P, Fasano F, Calabresi C, Cazzullo CL, Vergani C, Annoni G, Clerici M: Interleukin-10 and interleukin-6 gene polymorphisms as risk factors for Alzheimer's disease. *Neurobiol Aging* 2004;25:1009–1015.
- Licastro F, Porcellini E, Caruso C, Lio D, Corder EH: Genetic risk profiles for Alzheimer's disease: integration of APOE genotype and variants that up-regulate inflammation. *Neurobiol Aging* 2007;28:1637–1643.
- Li H, Wetten S, Li L, St Jean PL, Upmanyu R, Surh L, Hosford D, Barnes MR, Briley JD, Borrie M, Coletta N, Delisle R, Dhalla D, Ehm MG, Feldman HH, Fornazzari L, Gauthier S, Goodgame N, Guzman D, Hammond S, Hollingworth P, Hsiung GY, Johnson J, Kelly DD, Keren R, Kertesz A, King KS, Lovestone S, Loy-English I, Matthews PM, Owen MJ, Plumptre M, Pryse-Phillips W, Prinjsa RK, Richardson JC, Saunders A, Slater AJ, St George-Hyslop PH, Stinnett SW, Swartz JE, Taylor RL, Wherrett J, Williams J, Yarnall DP, Gibson RA, Irizarry MC, Middleton LT, Roses AD: Candidate single-nucleotide polymorphisms from a genome wide association study of Alzheimer disease. *Arch Neurol* 2008;65:45–53.
- Ravaglia G, Paola F, Maioli F, Martelli M, Montesi F, Bastagli L, Bianchin M, Chiappelli M, Tumini E, Bolondi L, Licastro F: Interleukin-1beta and interleukin-6 gene polymorphisms as risk factors for AD: a prospective study. *Exp Gerontol* 2006;41:85–92.
- Depboylu C, Lohmuller F, Gocke P, Du Y, Zimmer R, Gasser T, Klockgether T, Dodel RC: An interleukin-6 promoter variant is not associated with an increased risk for Alzheimer's disease. *Dement Geriatr Cogn Disord* 2004;17:170–173.

- 22 Bagli M, Papassotiropoulos A, Knapp M, Jensen F, Luise Rao M, Maier W, Heun R: Association between an interleukin-6 promoter and 3' flanking region haplotype and reduced Alzheimer's disease risk in a German population. *Neurosci Lett* 2000;283:109-112.
- 23 Zhang Y, Hayes A, Pritchard A, Thaker U, Haque MS, Lemmon H, Harris J, Cumming A, Lambert JC, Chartier-Harlin MC, St Clair D, Iwatsubo T, Mann DM, Lendon CL: Interleukin-6 promoter polymorphism: risk and pathology of Alzheimer's disease. *Neurosci Lett* 2004;362:99-102.
- 24 Koivisto AM, Helisalmi S, Pihlajamäki J, Moilanen L, Kuusisto J, Laakso M, Hiltunen M, Keijo K, Hanninen T, Helkala EL, Kervinen K, Kesaniemi YA, Soininen H: Interleukin-6 promoter polymorphism and late-onset Alzheimer's disease in the Finnish population. *J Neurogenet* 2005;19:155-161.
- 25 Van Oijen M, Arp PP, de Jong FJ, Hofman A, Koudstaal PJ, Uitterlinden AG, Breteler MM: Polymorphisms in the interleukin 6 and transforming growth factor beta1 gene and risk of dementia. The Rotterdam Study. *Neurosci Lett* 2006;402:113-117.
- 26 Capurso C, Solfrizzi V, Colacicco AM, D'Introno A, Frisardi V, Imbimbo BP, Lorusso M, Vendemiale G, Denitto M, Santamato A, Seripa D, Pilotto A, Fiore P, Capurso A, Panza F: Interleukin 6-174 G/C promoter and variable number of tandem repeats (VNTR) gene polymorphisms in sporadic Alzheimer's disease. *Prog Neuropsychopharmacol Biol Psychiatry* 2010;34:177-182.
- 27 Angelis P, Scharf S, Mander A, Vajda F, Christophidis N: Serum interleukin-6 and interleukin-6 soluble receptor in Alzheimer's disease. *Neurosci Lett* 1998;244:106-108.
- 28 Lanzrein AS, Johnston CM, Perry VH, Jobst KA, King EM, Smith AD: Longitudinal study of inflammatory factors in serum, cerebrospinal fluid, and brain tissue in Alzheimer disease: interleukin-1beta, interleukin-6, interleukin-1 receptor antagonist, tumor necrosis factor-alpha, the soluble tumor necrosis factor receptors I and II, and alpha1-antichymotrypsin. *Alzheimer Dis Assoc Disord* 1998;12:215-227.
- 29 Valente C, Maione P, Lippi A, et al: Validation of the Mini-Mental State Examination (MMSE) as a screening instrument for dementia in an Italian population. *Giorn Gerontol* 1992;40:161-5.
- 30 Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res* 1988;16:1215.
- 31 Wenham PR, Price WH, Blundell G: Apolipoprotein E genotyping by one-stage PCR. *Lancet* 1991;337:1158-1159.
- 32 Fishman D, Faulds G, Jeffery R, Mohamed-Ali V, Yudkin JS, Humphries S, Woo P: The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J Clin Invest* 1998;102:1369-1376.
- 33 Janka Z, Juhász A, Rimanóczy A A, Boda K, Márki-Zay J, Kálmán J: Codon 311 (Cys -> Ser) polymorphism of paraoxonase-2 gene is associated with apolipoprotein E4 allele in both Alzheimer's and vascular dementias. *Mol Psychiatry* 2002;7:110-112.
- 34 Raygani AV, Zahrai M, Raygani AV, Doosti M, Javadi E, Rezaei M, Pourmotabbed T: Association between apolipoprotein E polymorphism and Alzheimer disease in Tehran, Iran. *Neurosci Lett* 2005;375:1-6.
- 35 Pandey P, Pradhan S, Mittal B: Presenilin gene predisposes to late-onset degenerative but not vascular dementia: a comparative study of PS1 and ApoE genes in a North Indian Cohort. *Dement Geriatr Cogn Disord* 2007;24:151-161.
- 36 Luthra K, Tripathi M, Grover R, Dwivedi M, Kumar A, Dey AB: Apolipoprotein E gene polymorphism in Indian patients with Alzheimer's disease and vascular dementia. *Dement Geriatr Cogn Disord* 2004;17:132-135.
- 37 Pirttilä T, Lehtimäki T, Rinne J, Mattila K, Frey H, Nikkari T: The frequency of apolipoprotein E4 allele is not increased in patients with probable vascular dementia. *Acta Neurol Scand* 1996;93:352-354.
- 38 Pola R, Gaetani E, Flex A, Aloï F, Papaleo P, Gerardino L, De Martini D, Flore R, Pola P, Bernabei R: -174 G/C interleukin-6 gene polymorphism and increased risk of multi-infarct dementia: a case-control study. *Exp Gerontol* 2002;37:949-955.
- 39 Rao KM, Pieper CS, Currie MS, Cohen HJ: Variability of plasma IL-6 and cross linked fibrin dimers over time in community dwelling elderly subjects. *Am J Clin Pathol* 1994; 102:802-805.
- 40 Ershler WB, Keller ET: Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med* 2000;51:245-270.
- 41 Krabbe KS, Pedersen M, Bruunsgaard H: Inflammatory mediators in the elderly. *Exp Gerontol* 2004;39:687-699.
- 42 Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D: Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* 2004;292:1440-1446.
- 43 Kopp HP, Kopp CW, Festa A, Kryzanowska K, Kriwanek S, Minar E, Roka R, Schernthaner G: Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. *Arterioscler Thromb Vasc Biol* 2003;23:1042-1047.
- 44 Kaur G, Raptap CC, Kumar N, Kumar S, Neolia S, Mehra NK: Frequency distribution of cytokine gene polymorphisms in the healthy North Indian population. *Tissue Antigens* 2007;69:113-120.